AD_____

WORLD REFERENCE CENTER FOR ARBOVIRUSES

ANNUAL REPORT

ROBERT E. SHOPE

MARCH 8, 1992

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21702-5012

Contract No. DAMD17-90-Z-0020

Yale University School of Medicine
P.O. Box 3333
New Haven, Connecticut 06510

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3 DISTRIBUTION / AVAILABILITY OF REPORT Approved for public release; distribution unlimited
2b. DECLASSIFICATION / DOWNGRADING SCHEDULE	
4 PERFORMING ORGANIZATION REPORT NUMBER(S)	5 MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION Yale University School of Medicine 6b OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code) P.O. Box 3333 New Haven, Connecticut 06510	7b. ADDRESS (City, State, and ZIP Code)
8a. NAME OF FUNDING / SPONSORING ORGANIZATION U.S. Army Medical Research & Development Command 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER DAMD17-90-Z-0020
8c. ADDRESS (City, State, and ZIP Code) Fort Detrick Frederick, Maryland 21702-5012	10. SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO. 61102A; PROJECT NO. 3M1-61102BS13; TASK NO. AA; WORK UNIT ACCESSION NO DA331036

11. TITLE (Include Security Classification)
World Reference Center for Arboviruses

12. PERSONAL AUTHOR(S)
Robert E. Shope

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Annual	FROM 2/9/91 TO 2/8/92	1992, March 8	

16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	Arbovirus, Japanese encephalitis, dengue, Guanarito virus, Venezuelan hemorrhagic fever, vaccinia, RA 1
06	02		
06	03		

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

The major finding in 1991 was the discovery of a new arenavirus Guanarito, causative agent of Venezuelan hemorrhagic fever. The study of 15 patients in Portuguesa State, Venezuela confirmed the clinical syndrome of hemorrhagic fever, and the high case fatality rate (9/15). Serological diagnostic tests were developed including IgM capture ELISA. Initial observations in the field indicated a probable rodent-borne epidemiology. Other findings included a new genotype of Japanese encephalitis virus in Indonesia, and the development of recombinant vaccinia viruses excreting subviral particles that immunized mice to Japanese encephalitis and yellow fever viruses. The reference center distributed arboviral reagents to laboratories in the United States and in 9 other countries.

20. DISTRIBUTION / AVAILABILITY OF ABSTRACT	21 ABSTRACT SECURITY CLASSIFICATION	
☐ UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT ☐ DTIC USERS	Unclassified	
22a. NAME OF RESPONSIBLE INDIVIDUAL Mary Frances Bostian	22b TELEPHONE (Include Area Code) 301-619-7325	22c. OFFICE SYMBOL SGRD-RMI-S

DD Form 1473, JUN 86 *Previous editions are obsolete* SECURITY CLASSIFICATION OF THIS PAGE

TABLE OF CONTENTS

FOREWORD

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 86-23, Revised 1985).

For the protection of human subjects, the investigators have adhered to policies of applicable Federal Law 45CFR46.

INTRODUCTION

The World Reference Center for Arboviruses is supported jointly by the U.S. Department of Defense, the National Institutes of Health, and the World Health Organization. The Center identifies and characterizes suspected arboviruses submitted from U.S. and overseas laboratories, diagnoses disease outbreaks, develops new techniques for rapid diagnosis and for characterization of arboviruses, prepares and distributes reference immune reagents and specific nucleic acid probes, prepares virus stocks for distribution through WHO regional reference centers and the American Type Culture Collection, prepares and distributes antigens on a limited basis, carries out limited serological surveys, and disseminates information through WHO and the American Committee on Arboviruses.

Emphasis has been placed on specific subprojects including molecular epidemiology using primer extension analysis of flavivirus RNA, adaptation of ELISA for field application to arboviruses of human disease importance, use of the extensive WHO reagent bank for characterization of monoclonal antibodies, and engineering of vaccinia vectored flavivirus cDNA for immunization of domestic animals and man, and for diagnostic antigens.

I. INVESTIGATION OF AN OUTBREAK

A. Venezuelan hemorrhagic fever: Clinical and epidemiological observations; isolation of virus (R. Tesh, R. Salas, M. Wilson, and R. Rico-Hesse; studies in Venezuela were done by staff of the National Institute of Hygiene "Rafael Rangel"; Division of Transmissible Diseases, Venezuelan Ministry of Health; Miguel Oraa Hospital, Guanare; and Central University, Caracas)

An outbreak of severe hemorrhagic illness began in the municipality of Guanarito, Portuguesa State, Venezuela, in September 1989. It was initially thought to be dengue hemorrhagic fever. Subsequent detailed study of 15 cases confirmed the presence of a new viral disease, designated Venezuelan hemorrhagic fever. Cases of the disease have continued to be present, with a few patients seen each month. In the period from May 1, 1990 until March 30, 1991, a total of 104 presumed cases of the disease with 26 deaths were reported to the Ministry of Health. Most of the patients have been adults; to date, all have come from rural areas in the Municipality of Guanarito or from adjacent regions of neighboring Barinas State.

The Municipality of Guanarito is located in the central plains (llanos) of Venezuela, is about 2,481 km in total area, and comprises most of the southeastern portion of Portuguesa State. The climate is tropical with a mean annual temperature of 28 degrees C and annual rainfall of 1,300 mm. Most of the 24,000 inhabitants of the municipality live in rural zones and are involved in agriculture or cattle raising.

Fifteen cases of Guanarito virus infection were confirmed by virus isolation and/or antibody conversion between September 1990 and April 1991. Nine of these died. All the patients were rural residents of the Municipality of Guanarito. Characteristics of the illness were fever, toxicity, headache, arthralgia, diarrhea, conjunctivitis, pharyngitis, leucopenia (79%), thrombocytopenia (86%), and hemorrhagic manifestations. Other features included facial edema, cervical lymphadenopathy, nausea and vomiting, cough, chest or abdominal pain, and convulsions. The patients ranged in age from 6 to 54 years. The initial clinical impressions of the examining physicians of 14 patients were: viral syndrome (5), dengue hemorrhagic fever (4), classical dengue fever (1), viral hemorrhagic syndrome (2), lobas pneumonia (1), and convulsive syndrome (1). Virus was isolated from the spleen of a fatal case, as reported in the 1990 Yale Arbovirus Research Unit (YARU) report.

Nine (60%) of the 15 confirmed cases ultimately died, despite vigorous treatment with blood, fresh plasma, concentrated platelets, fibrinogen, vitamin K, intravenous fluids and electrolytes, antibiotics, oxygen and other supportive measures. Death occurred within 1 to 6 days after hospitalization. Autopsies were done on 6 of the fatal cases. Although their clinical courses varied, the patients had remarkably similar gross and histopathological autopsy findings. Generally the autopsy showed the following: (1) diffuse pulmonary edema and congestion with intraparenchymal and subpleural hemorrhages; (2) the liver was congested with focal hemorrhages and was yellow in color; (3) the heart was enlarged and showed punctiform epicardial hemorrhages; (4) the spleen was enlarged and congested; (5) the kidneys were edematous with loss of the

corticomedular border; and (6) blood was present in the stomach, large and small intestines, rectum, bladder and uterus.

The 6 surviving patients recovered without serious sequellae. One reported temporary alopecia and another had a mild hearing loss. Convalescence was prolonged; one survivor reported that he was very weak and unable to work for 2 months.

Guanarito virus was recovered from the sera and/or spleens from all of the fatal cases and from sera of 2 of the surviving patients. Specimens for culture were not obtained from the other 4 survivors; however, specific antibodies to the virus were detected in their convalescent sera and were absent in their acute phase sera. None of hte patients showed culture or serologic evidence of recent dengue virus infection.

Based on the epidemiologic characteristics of these cases, their clinical and laboratory features, and their association with a previously unrecognized arenavirus (Guanarito), the 15 patients appear to represent a new disease entity which we have designated "Venezuelan hemorrhagic fever" (VHF). Although VHF is quite similar in its clinical manifestations, laboratory findings, and pathology to Lassa fever and to Argentine and Bolivian hemorrhagic fevers, its etiology and currently recognized geographic distribution are distinct. To date, all recognized cases of the disease have come from rural areas of 2 states (Portuguesa and Barinas) in central Venezuela.

II. IDENTIFICATION AND CHARACTERIZATION OF VIRUS

A. Guanarito virus (R. Tesh, R. Shope, and S. Tirrell) Sera from acutely ill patients and tissue samples taken at autopsy from fatal cases were submitted to the National Institute ofHygiene in Caracas for virus isolation. Some of these samples were also examined at YARU. Clinical specimens for virus isolation were inoculated into cultures of C6/36 mosquito cells and Vero cells. After 7 to 10 days, spot slides of the cultures were prepared and examined for viral antigen by indirect fluorescent antibody test (IFAT), using dengue serotype-specific mouse monoclonal antibodies and a polyclonal hyperimmune mouse ascitic fluid made against Guanarito virus. Specimens were positive with the homologous antibody and negative with dengue antibodies.

The initial isolate, VINH95.551, made in September, 1990 was used for further serological studies. IFAT with Guanarito and 10 other New World arenaviruses (Table 1) showed broad cross-reaction with all new world arenaviruses except for LCM virus for which there was less cross-reaction.

A complement-fixation (CF) test was done with all of the described New World arenaviruses except for Machupo, for which antigen was not available. The results (Table 2) showed that Guanarito was most closely related to Amapari and Junin viruses. It was also quite closely related to Tacaribe virus, and much less so to the other arenaviruses. There was no CF cross-reaction of any of the New World arenaviruses with LCM virus.

A strain of Guanarito virus was isolated from spleen of Sigmodon hispidus captured in Guanarito during early 1991 from the house of a VHF patient. Another rodent, an Oryzomys, had IFAT antibody.

Table 1. Results of IFAT with LCM, Guanarito and 9 other New World arenaviruses

ANTIGEN	ANTIBODY										
	Guanarito	Junin	Machupo	Tacaribe	Amapari	Latino	Parana	Tamiami	Flexal	Pichinde	LCM
Guanarito	≥1280*	640	≥1280	640	640	640	1280	≥1280	≥1280	320	80
Junin	≥1280	320	640	80	1280	640	1280	640	640	640	80
Tacaribe	≥1280	1280	640	80	≥1280	320	640	320	640	640	80
Amapari	≥1280	80	320	320	640	320	640	320	160	640	10
Latino	≥1280	320	320	40	80	≥1280	640	160	320	40	10
Parana	≥1280	40	160	40	320	320	≥1280	320	640	≥1280	160
Tamiami	≥1280	40	80	80	40	160	≥1280	≥1280	320	320	40
Flexal	≥1280	320	160	640	640	1280	≥1280	1280	≥1280	≥1280	80
Pichinde	640	40	80	<10	80	160	1280	320	320	≥1280	10
LCM	40	<10	10	20	20	40	40	20	20	160	≥1280

*Reciprocal of highest positive antibody dilution. Antigens were infected Vero cells.

Table 2. Results of complement fixation with LCM, Guanarito and 8 other New World arenaviruses

ANTIGEN	ANTIBODY									
	Guanarito	Junin	Tacaribe	Amapari	Latino	Parana	Tamiami	Flexal	Pichinde	LCM
Guanarito	256/32*	16/32	8/8	16/32	32/32	32/128	0/0	0/0	8/8	0/0
Junin	128/128	64/512	16/64	16/32	256/128	256/128	0/0	32/32	8/8	0/0
Tacaribe	32/32	32/128	32/128	16/32	128/128	128/128	0/0	128/128	0/0	0/0
Amapari	128/128	16/128	8/128	64/128	128/128	256/128	0/0	32/32	8/8	0/0
Latino	8/2	0/0	8/2	8/2	>20480/8	128/2	0/0	8/2	8/2	0/0
Parana	0/0	0/0	0/0	0/0	64/8	>81920/128	0/0	64/8	16/8	0/0
Tamiami	0/0	0/0	0/0	0/0	32/512	512/>512	128/128	64/128	8/8	0/0
Flexal	8/8	0/0	8/8	0/0	128/32	10240/128	0/0	40960/32	32/32	0/0
Pichinde	16/2	0/0	0/0	0/0	128/2	10240/32	0/0	512/32	512/8	0/0
LCM	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	128/128

*Reciprocal of antibody/reciprocal of antigen titer.

B. Identification of a new genotype of Japanese encephalitis virus from Indonesia (W. Chen, R. Rico-Hesse, H. Kusnanto, and R. Tesh)

During 1991, 12 Japanese encephalitis strains from Indonesia were examined by limited primer extension sequencing to evaluate the genetic divergence among JE viruses and their relationship to other antigenically related flaviviruses in the region. Previously this technique had been applied to a wide geographic range of JE viruses, resulting in the definition of three distinct genotypes. The results this year indicate the existence of a fourth genotype.

The JaOArS982 strain which has been completely sequenced, was used as the prototype. The 12 strains from Indonesia had been isolated between 1977 and 1981 by the NAMRU detachment in Jakarta. All but one of these viruses were isolated in mosquito cells by the late James Converse as part of the Navy's study to define the mosquito-borne viruses of Indonesia. Strains came from the islands of Java, Bali, and Flores. Seven other JE strains from wide geographic areas of Asia were sequenced for comparison.

The viruses were propagated in mosquito cells and each was identified initially using mouse hyperimmune JE serum by CF or ELISA, then confirming with a JE monoclonal antibody by IFA.

The sequencing technique was described by Chen, Tesh, and Rico-Hesse (J. Gen. Virol. 71:2915-2922, 1990). Japanese encephalitis virus RNA sequences were determined using the dideoxynucleotide method modified for RNA templates by extension of a synthetic DNA primer with reverse transcriptase. Five different primers were used to obtain the JE virus nucleotide sequence information. The sequence of a 240-nucleotide region in the preM gene of all JE virus strains was determined by priming the reverse transcriptase with two synthetic DNAs, M-98 and M-99. For those Indonesian strains not primed with M-98 and M-99, then JE/809, a 22-mer, and JE/612, a 19-mer, were used. These numbers refer to the map sites on the cloned and sequenced prototype strain JaOArS982. Two of the Indonesian strains -- JKT9002 and JKT8442 -- were not primed for sequencing by any of the above synthetic DNAs; consequently, an additional primer, JE/580, a 23-mer was used.

Nucleic acid swquences of the pre-M gene region were obtained for each of the 19 strains. All sequences were compared with the reported nucleotide position 456-695 of the prototype JE virus. Differences between the prototype and the other JE virus strains occurred in 94 positions; these were fairly evenly distributed. Most (68 of 94) of these differences were transitions. Over the 240 nucleotides used for comparison, the 19 JE viruses differed from the prototype JaOArS982 by 10 to 54 nucleotides. Most changes were shared by more than two isolates. For example, 17 of 19 isolates had an A instead of a C substitution at position 674.

Of eleven Indonesian JE viruses, 7 were from Java, 3 from Bali, and one from Flores. All but one were from Culex tritaeniorhynchus mosquitoes. The other was from Anopheles vagus.

Eighty amino acids are encoded in the preM region; two to 14 amino acid sequence divergence was observed among the viruses examined. Fifteen of the JE virus isolates had less than six amino acid changes. Amino acid

sequence divergence between JE isolates was lower than nucleotide sequence divergence, since most nucleotide changes were silent. Many of the amino acid changes were observed in more than two isolates. Two isolates, JKT8442 and JKT9092, showed more divergence than others at amino acid position 181-185, but their polarity remained unchanged when compared to other JE isolates.

The nucleotide sequences of MVE, WN, and Kunjin viruses were compared with the prototype; the majority of the nucleotide substitutions were also found at the corresponding positions for the JE virus isolates. There were 77 nucleotide differences between MVE and the prototype JE; 36 of 77 were identical to those of the JE isolates. Kunjin virus showed 85 nucleotide differences from JE prototype within the region of comparison; 25 of these differences were identical to those of the JE isolates. Since WN virus was more divergent from the JE, MVE, and Kunjin viruses, it was not included further in the study.

There were 21 differences among JE virus strains; for 14 of 21 substitutions, the replacement amino acid was found at the corresponding position in either MVE, Kunjin, or WN viruses. There were 25 amino acid differences between MVE and the prototype JE virus; four of these differences also occurred in one or more of the remaining JE isolates. Twenty-eight amino acid differences were observed between WN and JaOArS982 viruses. Kunjin virus varied from JaOArS982 at 31 amino acid positions.

A pairwise comparison was made of 240 nucleotides from the 20 JE viruses as well as the representative MVE and Kunjin virus strains. The maximum divergence over the 240-nucleotide sequence of the 20 JE isolates was 21%. In these comparisons, we defined a JE virus genotypic group as virus isolates that showed no more than 12% genomic divergence within the nucleotide interval of 456-695. The first group included virus strains from Japan and the Philippines. Strains from Southeast Asia formed three distinct genotypic groups. A northern Thailand isolate was distinct from other JE virus isolates at the 12% genomic divergence limit, and represents a separate genotypic group. Seven Indonesian virus isolates, as well as one each from southern Thailand, Malaysia, and Sarawak fell into the same cluster. Five Indonesian isolates were similar to each other and distinct from the other Indonesian isolates. MVE showed a 30% genomic divergence from the 20 JE virus isolates, while Kunjin virus showed a 36% difference. Thus, MVE virus appeared to be closer related to JE virus than to Kunjin or WN viruses by comparison of a short nucleotide sequence.

Two distinct nucleotide sequence divergence patterns were seen among the 11 Indonesian virus isolates. Most of the isolates from Java and one from Bali formed one genotypic group that had a 12% sequence divergence when compared with the JaOArS982 reference strain. These isolates correspond to the previously described second group. The remainder of Indonesian isolates, two from Java, two from Bali, and one from Flores formed another distinct genotypic group. This fourth group showed a 21% sequence divergence when compared with all other JE isolates, indicating that it was significantly different. The origin of the viruses did not appear to play an important role in determining their gen otype, since all but one of the Indonesian JE virus isolates were from Cx. tritaeniorhynchus mosquitoes.

III. SEROSURVEY OF HUMAN RESIDENTS OF SAO PAULO STATE FOR HANTAVIRUS ANTIBODY (B. Fonseca, A. Smith)

One hundred human sera from Sao Paulo, Brazil were referred by the Instituto Adolfo Lutz, Sao Paulo. These sera were collected during 1990-91 from patients with illness resembling leptospirosis, many of the patients showing hemorrhagic manifestations. They were from two different regions of Sao Paulo State, one on the coast, near a large and busy harbor (21% of samples), and the other a large urban area, the city of Sao Paulo which has slums and a large rat population.

Sera were tested at the 1:10 dilution by IFA using spot slides of Vero E6 Hantaan virus-infected cells (ROK 76-118). One human serum from Sao Paulo City was strongly positive and this result was repeated consistently. The finding of hantavirus antibody in the city of Sao Paulo is consistent with results of serosurvey of domestic rats by LeDuc et al. (Am. J. Trop. Med. Hyg. 34:810-815, 1985). It is planned to follow-up this finding with further attempts to define clinical disease and to isolate virus from rodents and patients.

IV. DEVELOPMENT OF NEW TECHNIQUES

A. Mice immunized with a sub-viral particle containing the Japanese encephalitis virus M and E proteins are protected from lethal JEV infection (E. Konishi, S. Pincus, E. Paoletti, R. Shope, and P. Mason)

The protection of mice immunized with recombinant vaccinia viruses containing Japanese encephalitis virus (JEV) cDNA constructs was described in the 1990 Yale Arbovirus Research Unit report. In order to determine if this protection was a result of subviral particles excreted by the recombinant vaccinia, these particles were purified away from the vaccinia virus and used directly as immunogens.

HeLa cells were infected with vP829, encoding prM and E. Extracellular particles were purified by ultracentrifugation of infected cell culture fluid. Particles were visualized by EM (T. Burrage, USDA, Plum Island, Greenport NY). The particles were then filtered and fractionated on a 10-35% continuous sucrose density gradient. The fraction showing the maximum HA activity was used for immunizatior of the mice. The purity of the fraction was confirmed by sodium dodecyl sulfate-containing polyacrylamide gel electrophoresis, and the E protein was quantified based on intensity of staining with Coomassie brilliant blue using bovine serum albumin as a standard. The presence of the M and E proteins in the extracellular particles was proved by the direct enzyme-linked immunosorbent assay using monoclonal antibodies against the JEV M (J2-2F1) and E (J3-11B9) proteins.

Vaccinia viruses that were not removed from culture fluids during the pruification process were inactivated by incubating hte fraction at 28C for 6 hr with freshily prepared binary ethylenimine (BEI) at a final concentration of 0.06%. The BEI was neutralized with sodium thiosulfate and the fraction was used to immunize mice, with and without Freund's complete adjuvant.

Groups of 3-week old outbred Swiss mice were inoculated as shown in Table 3. Three weeks later, sera were collected and mice were challenged by intraperitoneal injection with 0.1 ml of a 1:10 dilution of the brain homogenate from suckling mice infected with the Beijing P3 strain of JEV (4.9 x10/5 LD50). Following challenge, mice were observed daily for 3 weeks.

The plaque reduction neutralization and HI antibody titers in sera pooled from all mice prior to challenge showed that 10 ug of the E protein elicited levels of antibody similar to those elicited by 10/7 PFU of vP829.

Survival data were consistent with the serological data (Figure 1). Mice immunized with 10 ug of E were fully protected from the lethal JEV challenge.

That purified JE virus subunits protect mice against lethal challenge suggests that particles may provide the key ingredient in the protective immune response. It is possible that these subviral particles could be used directly as a vaccine.

B. Yellow fever recombinant vaccinia viruses (B. Fonseca, P. Mason, E. Konishi, S. Pincus, and E. Paoletti)

Vaccinia stocks were produced in Vero or MRC-5 cells. The 17D vaccine strain of yellow fever (YF) virus was grown in C6/36 cells. Mice were challenged with the French neurotropic YF strain in vaccination experiments. Vaccinia recombinants were produced at Virogenetics Laboratories, Troy, NY. The resultant recombinants were designated vP764 and vP869. vP869 contains a cDNA encoding 21 aa of C, prM, E, NS1, and NS2A. vP764 contains the same genomic regions plus the remainder of C. vP457 is the wild type vaccinia.

Synthesis of E and NS1 proteins in recombinant vaccinia viruses and YF 17D were compared. Proteins made by YF and by recombinant vaccinia viruses were immunoprecipitated by their respective monoclonal antibodies. When tested by gel electrophoresis, they migrated in an unusual fashion, indicating that they were not properly processed and expressed by the recombinant vaccinia viruses. vP869, however, did show hemagglutination of red cells, indicating probably that it secretes subviral particles.

The ability of the recombinant vaccinia viruses to protect mice against i.c. YF challenge was tested. Three-week old Swiss mice were immunized with a single i.p. dose of either vP764 and vP869. There were 20% surviving mice in the normal vaccinia control and in the vP764 recombinant immunized mice. On the other hand, vP869 conferred 90% protection and 17D vaccine conferred 50% protection.

Table 3. Immunizing conditions and antibody titers in mice.

Material for injection[a]	Amount per mouse[b]	Use of Adjuvant[c]	Injection Rout[d]	No. of mice	Antibody titer NEUT[e]	Antibody titer HAI[f]
Particle	10 ug	+	s.c.	4	1:80	1:40
Particle	1 ug	+	s.c.	5	1:20	1:10
Particle	1 ug	-	i.p.	5	1:10	<1:10
Buffer		+	s.c.	5	<1:10	<1:10
vP829	1.0×10^7 PFU	-	i.p.	5	≥1:80	1:20
vP829	3.3×10^2 PFU[g]	-	i.p.	5	<1:10	<1:10

[a]Groups of 3-week-old male mice were immunized with the purified extracellular particles (Particle) or a recombinant vaccinia virus (vP829), or injected with 10 mM Tris-HCl (pH 7.5) containing 100 mM NaCl used for suspending the purified extracellular particles (Buffer).

[b]Represented as amounts of the E protein for the purified extracellular particles or as infective titers for vP829.

[c]Freund's complete adjuvant.

[d]Subcutaneous (s.c.) or intraperitoneal (i.p.).

[e]Serum dilution yielding 90% reduction in plaque number (refer to 1).

[f]Serum dilution.

[g]The amount of vaccinia viruses contained in the purified extracellular particle fraction corresponding to 10 ug of E before inactivation with BEI.

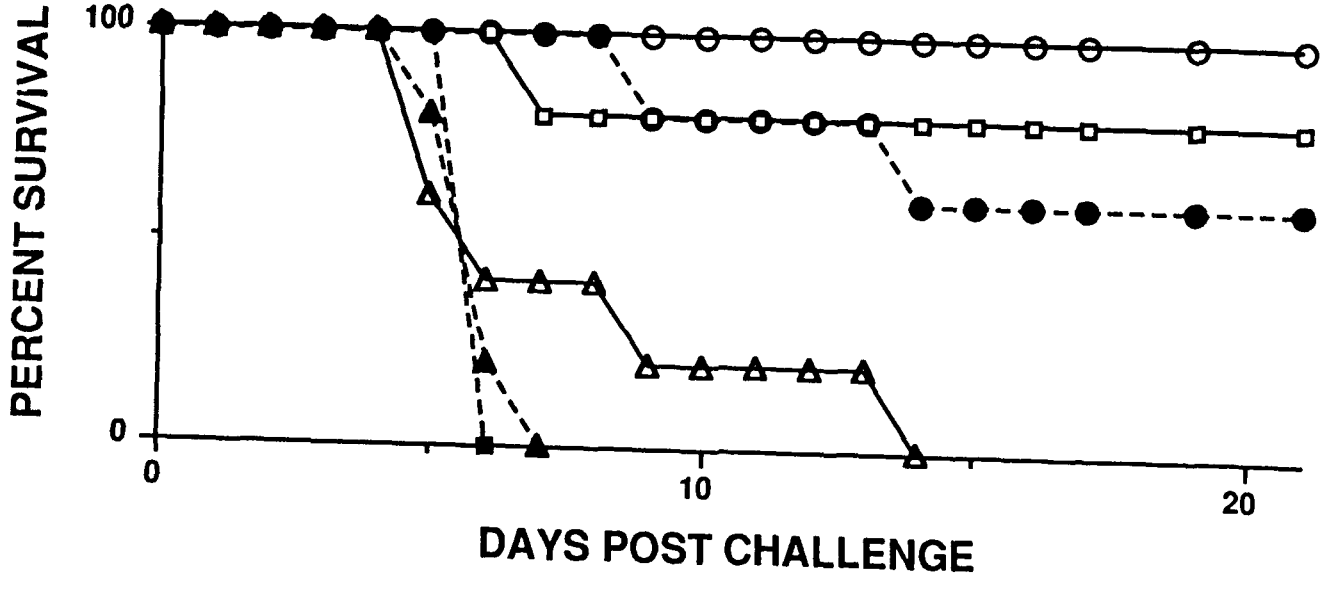

Figure 1. Survival of mice inoculated once with purified extracellular JEV particles. Particles contained 10 ug E plus FCA (open circle), 1 ug E with FCA (closed circle) or no FCA (open triangle), FCA alone (closed triangle), 10/7 pfu of vP829 (open square), or 3.3x10/2 pfu of vP829 (closed square) prior to challenge with 4.9x10/5 LD50 of JEV.

V. DISTRIBUTION OF REAGENTS (R. Shope, S. Tirrell, R. Tesh)

Reagents were distributed in 56 shipments to laboratories in 10 different countries (USA, UK, France, Trinidad, Israel, Venezuela, Germany, Spain, Senegal, and Nigeria). Distribution included 94 ampoules of virus, 68 of antibody, 10 of antigen, and one of RNA, as well as shipments of live sand flies, kissing bugs, and C6/36 mosquito cells.

VI. CONCLUSIONS

The major finding in 1991 was the discovery of a new arenavirus Guanarito, causative agent of Venezuelan hemorrhagic fever. The study of 15 patients in Portuguesa State, Venezuela confirmed the clinical syndrome of hemorrhagic fever, and the high case fatality rate (9/15). Serological diagnostic tests were developed including IgM capture ELISA. Initial observations in the field indicated a probable rodent-borne epidemiology. Other findings included a new genotype of Japanese encephalitis virus in Indonesia, and the development of recombinant vaccinia viruses excreting subviral particles that immunized mice to Japanese encephalitis and yellow fever viruses. The reference center distributed arboviral reagents to laboratories in the United States and in 9 other countries.

VII. PUBLICATIONS

Barry, M., Patterson, J.E., Tirrell, S., Cullen, M.R., and Shope, R.E. The effect of chloroquine prophylaxis on yellow fever vaccine antibody response: Comparison of plaque reduction neutralization test and enzyme-linked immunosorbent assay. Am. J. Trop. Med. Hyg. 44:79-82, 1991.

Brown, S.E., Morrison, H.G., Karabatsos, N., and Knudson, D.L. Genetic relatedness of two new Orbivirus serogroups: Orungo and Lebombo. J. Gen. Virol. 72:1065-1072, 1991.

Chapman, L.E., Wilson, M.L., Hall, D.B., LeGuenno, B., Dykstra, E.A., Ba, K., and Fisher-Hoch, S.P. Risk factors for Crimean-Congo hemorrhagic fever in rural northern Senegal. J. Inf. Dis. 164:686-692, 1991.

Gonzalez, J.P., Camicas, J.L., Cornet, J.P., Faye, O., and Wilson, M.L. Sexual and transovarial transmission of Crimean-Congo haemorrhagic fever virus in Hyalomma truncatum. Res. Virol. 143:23-28, 1992.

Gonzalez, J.P., Cornet, J.P., Wilson, M.L., and Camicas, J.L. Crimean-Congo hemorrhagic fever virus replication in adult Hyalomma truncatum and Amblyomma variegatum ticks. Res. Virol. 142:483-488, 1991.

Greiser-Wilke, I.M., Moennig, V., Kaaden, O.-R., and Shope, R.E. Detection of alphaviruses in a genus-specific antigen capture enzyme immunoassay using monoclonal antibodies. J. Clin. Microbiol. 29:131-137, 1991.

Howe, D.K., Vodkin, M.H., Novak, R.J., Shope, R.E., and McLaughlin, G.L. Use of the polymerase chain reaction for the sensitive detection of St. Louis encephalitis viral RNA. J. Virol. Methods 36:101-110, 1991.

Konishi, E., Pincus, S., Fonseca, B.A.L., Shope, R.E., Paoletti, E., and Mason, P.W. Comparison of protective immunity elicited by recombinant vaccinia viruses that synthesize E or NS1 of Japanese encephalitis virus. Virology 185:401-410, 1991.

Mason, P.W., Pincus, S., Fournier, M.J., Mason, T.L., Shope, R.E., and Paoletti, E. Japanese encephalitis virus-vaccinia recombinants produce particulate forms ofthe structural membrane proteins and induce high levels of protection against lethal JEV infection. Virology 180:294-305, 1991.

Salas, R., Manzione, N.D., Tesh, R.B., Rico-Hesse, R., Shope, R.E. et al. Venezuelan haemorrhagic fever. Lancet 338:1033-1036, 1991.

Shope, R.E. Global climate change and infectious diseases. Environ. Hlth. Perspectives 96:171-174, 1991.

Wilson, M.L., Gonzalez, J.P., Cornet, J.P., and Camicas, J.L. Transmission of Crimean-Congo haemorrhagic fever virus from experimentally infected sheep to Hyalomma truncatum ticks. Res. Virol. 142:395-404, 1991.

ARTICLE IN PRESS

Wilson, M.L. and Deblinger, R.D. Vector management to reduce the risk of Lyme disease. In: Ecology and Environmental Management of Lyme Disease.

Ginsberg, H.S. (ed.). New Brunswick, NJ: Rutgers University Press.

ARTICLES SUBMITTED

Keyes, L.E., Wilson, M.L., and Gonzalez, J.P. Experimental infection with Crimean-Congo hemorrhagic fever virus using laboratory mice. Res. Virol.

Wilson, M.L., Dykstra, E.A., and Schmidt, E.A. Temperature- and humidity-dependent longevity of unfed adult Hyalomma truncatum (Acari: Ixodidae). J. Med. Entomol.

Durden, L.A., Logan, T.M., Wilson, M.L., and Linthicum, K.J. Vector incompetence of a soft tick, Ornithodoros sonrai for Crimean-Congo hemorrhagic fever virus under laboratory conditions. J. Inf. Dis.

Deblinger, R.D., Wilson, M.L., Rimmer, D.W., and Spielman, A. Reduced abundance of deer ticks, Ixodes dammini (Acari: Ixodidae) following incremental removal of deer density. J. Med. Entomol.